Prestin belongs to the SLC26 protein family of anion transporters. Prestin (SLC26A5) is a motor protein essential for the electromotility of the outer hair- cells (OHC) and therefore the amplification of sound in the cochlea. This protein is able to convert changes in membrane potential to mechanical force and to modify the length of the OHC. The electromotility of prestin-expressing cells is associated with a nonlinear capacitance (NLC) that can be measured electrophysiologically. Prestin is a mechanosensitive protein and its function is altered by reagents known to change membrane mechanical properties.
The non-steroidal anti-inflammatory drug (NSAID) salicylate has been showed to inhibit the NLC and the electromotility of OHC and prestin-expressing HEK. It is thought to compete with anions such as chloride for the anion-binding site on prestin. Other NSAIDs such as ibuprofen, naproxen, piroxicam or diflusinal can trigger side effects related with hearing and sometime cause tinnitus by an unknown mechanism. Here, we investigate a possible mechanism for these adverse reactions by examining the effects of these drugs on the function of prestin. The prestin-associated NLC is monitored by whole-cell as well as inside-out patches from HEK cells expressing prestin, and recorded before and after the perfusion of NSAIDs. This allows a cell-by-cell comparison of the NLC parameters in the presence and absence of the tested molecule. Ibuprofen and naproxen both showed an effect on the half-maximum voltage (V1/2) and the charge density parameters of the NLC. The effect of ibuprofen was particularly pronounced, shifting V1/2 from −70 mV to −52 mV and increasing the maximal charge movement by 30%. Further studies will contribute to our understanding of whether NSAIDs act through alteration of the mechanical properties of the membrane or specifically interfere with prestin function.

3325-Pos Board B186
Thermal Sensitivity of Vestibular Neuroepithelium in the Toadfish
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Core body temperature is known affect the static and dynamic properties of vestibular nerve afferents, although the underlying mechanisms for the differential sensitivity of the two afferents types to temperature changes are unknown. We recorded the changes in spontaneous discharge rate, regularity, adaptation to step hair bundle displacements and responses to sinusoidal head rotations of single-units to changes in temperature of the crista ampullaris in the oyster toadfish. Similar temperature gradients were introduced with perfusion of temperature-controlled artificial perilymph and with irradiation of the crista with 980 and 1860 nm light. A broad spectrum of afferent responses to thermal perfusion was observed: some neurons were insensitive to temperature, some increased their spontaneous discharge rate, and others showed a decrease in their discharge rate with temperature. The very short focused pulses of light at the two wavelengths deliver transient pulses of thermal energy (dT/dt) without large increases in accumulated temperature of cells or tissue. The irradiation of the crista produced different responses from the same afferents for a similar change in temperature. These results suggest potential wavelength specific absorption within the vestibular neuroepithelium underlies the differences between the three forms of heat delivery. Depending upon the hair cell type irradiated, the fast temperature changes induced may modulate the intracellular Ca2+ levels differently affecting the neurotransmitter release. Regardless of the mechanisms involved, the presence of temperature compensating mechanisms in the hair cell and afferent complex may allow sensitive function over a wide range of temperatures.

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Imaging Stereocilia Links in Live Auditory Hair Cells
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We previously imaged stereocilia links in glutaraldehyde-fixed mammalian auditory hair cells using hopping probe scanning ion conductance microcopy (HPSICM, Novak *et al*. Nat Methods, 2009). Due to the complex three-dimensional structure of the stereocilia bundle, achieving high-resolution images required a considerable amount of time (~44 min/bundle). To study the dynamics of stereocilia link regeneration in real time, we needed a significantly faster yet reliable way to continuously scan the stereocilia bundles in live hair cells.
To improve the HPSICM imaging speed, we re-designed the scan head by incorporating a faster piezo assembly with a resonant frequency of ~18 kHz for Z-movement. Despite having a less sensitive strain gauge sensor (compared to the previously used capacitive sensor), the vertical resolution of the system remained the same (~5nm). Significantly smaller inertia allowed mounting the scan head on a rotational platform and scanning the sample at any angle, a pre-requisite for the successful imaging of tip links. Moreover, the overall image resolution was slightly decreased and we can now image hair cell bundles significantly faster (~11 min/bundle).
The performance of the improved system was tested using cultured organ of Corti explants from the *Shaker 2* and *Whirler* mice due to their short stereocilia with abundant stereocilia links (typically ~5nm in diameter and ~100-300nm in length). To test the ability of HPSICM to detect these miniature structures at high imaging speed we performed continuous time-lapse scanning and looked for reproducibility of the links in consecutive images. Next, to rule out the possibility that the observed links were simply scanning artifacts, we disrupted the links by treating the explants with BAPTA-buffered Ca^{2+}-free medium. Our results demonstrate that the improved HPSICM technique successfully visualizes stereocilia links in live auditory hair cells.
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Nonlinearities in Threshold-Level Detection by Inner Ear Hair Cells
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Auditory system is known for its exquisite sensitivity with sub-nanometer detection thresholds. Mechanical deflections due to external sound and ground vibrations are converted by inner ear hair cells into electrical signals. In some species, hair cell's stereociliary bundles exhibit spontaneous oscillations under *in vitro* conditions, a behavior that has been successfully reproduced by numerical models based on nonlinear dynamics. In this work, we study both numerically and experimentally the dynamics of individual hair bundles from the Bullfrog sacculus, at threshold levels of stimulation to elucidate the mechanisms underlying the sensitivity of detection by the sacculus. We measure the steady state response to small sinusoidal stimuli, as well as the time course of the phase locking transition in spontaneously oscillating bundles.

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Self-Tuning of Hair Cells in the Bullfrog Sacculus
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Spontaneous oscillations of the stereociliary bundle of a hair cell - the mechanosensory cell in auditory and vestibular systems - is considered to be a signature of an active amplification mechanism. We study whether an internal self-tuning process governs the active motility, by mimicking the effects of loud sound on the spontaneous oscillation. After the application of high-amplitude stimuli, with deflections on the order of micrometers applied to the hair bundle, the active oscillatory motion of the hair bundle was suppressed for hundreds of milliseconds, indicating a change in the dynamic state of the hair cell. Here we observe the recovery profile of an oscillating hair bundle after cessation of deflection. Data is compared to mathematical models which include a feedback equation to capture the temporal changes in the profile of the limit cycle oscillations.

Peptide & Toxin Ion Channels

3329-Pos Board B190
Increase in Cytotoxic Effect of Tolaasin by Phospholipids Composed of Medium-Chain Fatty Acids
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Tolaasin produced by *Pseudomonas tolaasii* disrupts membrane structures of cultivated mushrooms, such as *Agaricus bisporus* and *Pleurotus ostreatus* (oyster mushroom), and causes brown blotch disease. It consists of 18 amino acids, its molecular mass is 1,985 Da, and it forms a left-handed α-helix. The mechanism of membrane-pore formation of tolaasin molecule has not known in detail. When tolaasin molecule is inserted into the membrane, N-terminus of tolaasin forms 4 turns of α-helix and the length of tolaasin channel corresponds to near 20 Å, a little shorter than the thickness of membrane. Tolaasin channels are unstable in the artificial lipid bilayer and this may be explained by the comparison between the length of tolaasin channel and the thickness of lipid bilayer membrane. In control condition, bilayer was made with phosphatidyl